INFLUENCE OF DIFFERENT INOCULUM LEVELS OF *MELOIDOGYNE INCOGNITA*, *NECTRIA HAEMATOCOCCA* F. SP. *PIPERIS* AND *PHYTOPHTHORA PALMIVORA* ON BLACK PEPPER PLANTS*

FRANCISCO C.O. FREIRE[1] & JOHN BRIDGE

[1] EMBRAPA, CPATU, Caixa Postal 48, 66000 Belém,PA – Brazil
[2] COMMONWEALTH INSTITUTE OF PARASITOLOGY, 395 A Hatfield Road, St. Albans, Herts AL4 OXU U.K.

(Accepted for publication in 07/05/1985)

ABSTRACT

FREIRE, F.C.O. & BRIDGE, J. Influence of different inoculum levels of *Meloidogyne incognita, Nectria haematococca* f. sp. *piperis* and *Phytophthora palmivora* on black pepper plants. Fitopatol. bras. 10:559-575. 1985.

Meloidogyne incognita was found to be highly pathogenic to black pepper when inoculated at the inoculum levels of 100, 500, 1,000, 5,000 and 10,000 second-stage juveniles per seedling. Stunting and reduction of the number of leaves, reduction of the top fresh weight and of the total fresh weight of seedlings were noticed with increase in inoculum levels. The root-knot index and the final nematode population per gram of root revealed a steadily increasing trend with increase in inoculum levels. Among the fourteen inoculum levels of *Nectria haematococca* f. sp. *piperis* applied to roots of black pepper seedlings, those corresponding to 4×10^3, 3×10^3, 2×10^3 and 10^3 conidia neither produced wilt nor death of inoculated seedlings. The fungus was reisolated from plants inoculated with 4×10^3 and 3×10^3 conidia but not from plants inoculated with 2×10^3 and 10^3 conidia. The threshold inoculum level of *Nectria haematococca* f. sp. *piperis* capable of causing death of black pepper seedlings was 5×10^3 conidia. The dilution end point of this fungus was reached at the inoculum level of 3×10^3 conidia per seedling, below which the fungus reisolation was not achieved. The period between fungus inoculation and wilt symptons and the period between fungus inoculation and death of plants were shorter for seedlings inoculated with higher conidial concentrations. As for *Phytophthora palmivora* seventeen inoculum levels were applied to roots of black pepper seedlings. The period between fungus inoculation and wilt symptoms and the period between fungus inoculation and death of plants did not present great variations when all different inoculum levels were compared. These periods, however, were shorter for seedlings inoculated with higher zoosporic concentrations. The threshold inoculum level of *Phytophthora palmivora* capable of causing death of black pepper seedlings and the dilution end point below which the fungus could not be reisolated was 5 x 10^2 zoospores. Seedlings inoculated with 4 x 10^2, 3 x 10^2, 2 x 10^2 and 10^2 zoospores remained symptomless. Percentages of *Phytophthora palmivora* reisolation from symptomless plants were low compared to *Nectria haematococca* f. sp. *piperis*.

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* Portion of a Ph.D. thesis submitted by the senior author to London University.

RESUMO

Influência de Diferentes Níveis de Inóculo de *Meloidogyne incognita, Nectria haematococca* f. sp. *piperis* e *Phytophthora palmivora* em relação a Mudas de Pimenta-do-Reino

Meloidogyne incognita quando inoculado nos níveis de 100, 500, 1.000, 5.000 e 10.000 juvenis de segundo estágio por planta revelou-se extremamente patogênico a mudas de pimenta-do-reino. Todos os parâmetros avaliados, tais como, número de folhas por planta, altura das plantas, índice de galhas, peso fresco da parte aérea, peso fresco do sistema radicular e população final do nematóide por grama de raíz apresentaram diferenças significativas entre os tratamentos. Os efeitos negativos decorrentes do parasitismo do nematóide foram mais acentuados à medida que os níveis de inóculo aumentaram. Dos 14 níveis de inóculo de *Nectria haematococca* f. sp. *piperis* aplicados às raízes de mudas de pimenta-do-reino somente aqueles correspondendo a 4 x 10^3, 3 x 10^3, 2 x 10^3 e 10^3 conídios não produziram a murcha e a morte das plantas inoculadas. Entretanto, o fungo foi reisolado a partir de plantas inoculadas com 4 x 10^3 e 3 x 10^3 conídios, mas não daquelas inoculadas com 2 x 10^3 e 10^3 conídios. O nível de inóculo de 5 x 10^3 conídios foi a concentração mínima capaz de matar as plantas inoculadas. Os períodos entre a inoculação e o surgimento dos primeiros sintomas de murcha e entre a inoculação e a morte das plantas foram menores para as plantas inoculadas com maiores níveis de inóculo. Dezessete níveis de inóculo foram testados com relação a *Phytophthora palmivora*. O período decorrido entre a inoculação das mudas de pimenta-do-reino e o surgimento dos sintomas de murcha e entre a inoculação e a morte das mudas não apresentaram grandes variações entre os diferentes níveis de inóculo. O mais baixo nível de inóculo de *Phytophthora palmivora* capaz de causar a morte das plantas inoculadas foi 5 x 10^2 zooósporos. Os níveis correspondentes a 4 x 10^2, 3 x 10^2, 2 x 10^2 e 10^2 zooósporos não provocaram murcha ou morte das plantas. O reisolamento de *Phytophthora palmivora* a partir de plantas sem sintomas aparentes de infecção ocorreu numa percentagem menor comparado ao reisolamento de *Nectria haematococca* f. sp. *piperis*. O reisolamento de *Phytophthora palmivora* a partir de mudas inoculadas somente foi obtido até o nível de inóculo equivalente a 5 x 10^2 zooósporos por planta. Abaixo desta concentração o fungo não pôde ser reisolado.

INTRODUCTION

A considerable amount of information has accumulated on the occurrence and importance of plant pathogens in black pepper. Root-knot nematodes were first recorded in roots of this plant in 1901 by Zimmerman and in 1904 by Breda de Haan, in Java, Indonesia (cited by Holliday & Mowatt, 1963). Other reports on the occurrence of *Meloidogyne* spp. in roots of black pepper were also provided by Delacroix (1902) in Cochin-China, and by Butler (1906) in Wynad, Kerala, India (cited by Sundararaju et al., 1979). Since these early records various other workers have stressed the importance of *Meloidogyne* parasitism upon the health of black pepper in different countries (Ayyar, 1926; Nadakal, 1964; Venkitesan, 1972; Wimoto, 1972; Lordello & Silva, 1974; Sharma & Loof, 1974; Ichinohe, 1975; Freire & Monteiro, 1978; Jacob & Kurian, 1979; Ferraz & Sharma, 1979).

Pathogenicity tests have been conducted using as inocula second-stage juveniles and eggs of *Meloidogyne* spp. to demonstrate the serious damage that can be caused by these nematodes to black pepper plants. However, the relationships between the different inoculum levels of the nematode and their effects on the development of black pepper have received meagre attention, and apparently the only report that actually shows these relationships was provided by Kosky et al. (1979). Increase of damage to different species of plants as a consequence of increase in the levels of inoculum used, have been reported by many workers (e.g. Elgin et al., 1973; Wong & Mai, 1973; Dhawan & Sethi, 1976; Raut & Sethi, 1980; Raut, 1980; Reddy, 1981; Arens & Rich, 1981).

Root and foot rot of black pepper plants caused by *Nectria haematococca* f. sp. *piperis* is, to date, restricted to Brazil where it was first described (Albuquerque, 1961). Despite being an extremely pathogenic fungus to black pepper in the Brazilian Amazonian region, there has been no report concerning the inoculum density-disease incidence relationship for fungal inoculation in the root system.

Occurrence of *Phytophthora palmivora* as the causative agent of root and foot rot of black pepper was reported as early as 1936 by Muller in Indonesia, although this fungus had been isolated from pepper in India in the 1920s, but was not considered pathogenic by Venkata Rao (1929) (cited by Nambiar & Sarma, 1977). *P. palmivora* was reported affecting black pepper in Brazil relatively recently (Holliday, 1965; Albuquerque, 1966). Pathogenicity tests involving this fungus on different cultivares and species of black pepper, including some wild species, have been reported by several workers (Holliday & Mowatt, 1963; Ruppel & Almeyda, 1965; Turner 1971, 1973). None of these workers have determined the relationships between the inoculum levels of *P. palmivora* and the incidence of root and foot rot and mortality in plants of black pepper. Several workers have established such relations for many other different species of *Phytophthora* and plants, using chlamydospores, oospores, sporangia and zoospores as inocula (Gooding & Lucas, 1959; Kliejunas & Ko, 1974; Ramirez & Mitchell, 1975; Shew & Benson, 1979; Kannwischer & Mitchell, 1981).

The enormous economic importance of black pepper for the Brazilian Amazonian region, where this spice plant is the main cash crop, and the necessity of long-term control measures through the use of genetic resistance, make indispensable the utilization of extensive screening trials. For such tests, appropriate inoculum levels are extremely useful to evaluate properly the plant's response to the pathogens. The aim of this study was to find out accurate disease threshold levels of *M. incognita, N. haematococca* s. sp. *piperis* and *P. palmivora* applicable to black pepper plants and try to establish reliable relationships between the inoculum levels and the plant's reaction to these three pathogens.

MATERIALS AND METHODS

M. incognita – Six month-old black pepper seedlings, cv. Singapura, were grown in 10 cm plastic pots, partially filled with an autoclaved mixture of loam and sand (3:2) and inoculated with 100, 500, 1,000, 5,000 or 10,000 freshly hatched second-stage juveniles of *M. incognita.* Controls were represented by plants without nematodes. After inoculation, plants were kept in a heated glasshouse where temperatures ranged from 24ºC to 37ºC. Pots were arranged in a randomized complete block design and each treatment was replicated five times. Phostrogen fertilizer was added to the pots monthly at a concentration of 1g/litre of water.

Before inoculation with the nematodes, the height and the number of leaves of each plant were recorded. Six months after inoculation, plants were carefully uprooted, their root systems washed in tap water, and the following parameters assessed: final plant height, final number of leaves per plant, top fresh weight, root fresh weight, total fresh weight, root-knot index, number of females without eggs/g root, number of females with eggs/g root, total female population/g root and number of eggs/g root. N. haematococca f. sp. piperis – Eight month-old black pepper seedlings, cv. Singapura, were grown under the same conditions described for *M. incognita.* Each seedling was inoculated with 10^3, 2 x 10^3, 3 x 10^3, 4 x 10^3 5 x 10^3, 10^4, 2 x 10^4, 3 x 10^4, 4 x 10^4, 5 x 10^4, 10^5, 5 x 10^5, 10^6 or 5 x 10^6 conidia of *N. haematococca* f. sp. *piperis.* The fungus was cultured on peptone-potato dextrose agar medium (Peptone 10g; PDA Oxoid 39g; distilled water 1,000 ml). Inoculation was achieved by pouring the desirable amount of inocula (a suspension of macroconidia and microconidia) into three holes, around the plant, and filling the holes with soil again. Controls were represented by plants non-inoculated with the fungus and receiving only sterilized distilled water. Plants were kept in a heated glasshouse under the conditions previously described and were arranged in a randomized complete block design, each treatment being replicated five times.

Observations were made daily, over a four-month period, and the appearance of wilt symptoms or any other abnormality was recorded. The parameters evaluated were: period between fungus inoculation and wilt symptoms, period between fungus inoculation and plant death, percentage of plants wilted and dead and percentage of fungus isolation from wilted and symptomless plants.

P. palmivora – Eight month-old black pepper seedlings, cv. Singapura, were prepared as mentioned before. Each seedling was inoculated with 10^2, 2 x 10^2, 3 x 10^2, 4 x 10^2, 5 x 10^2, 10^3, 2 x 10^3, 3 x 10^3, 4 x 10^3, 5 x 10^3, 10^4, 2 x 10^4, 3 x 10^4, 4 x 10^4, 10^5 or 5 x 10^5 zoospores of *P. palmivora.* The fungus was cultured on V-8 juice agar medium (Campbell's V-8 juice, 200 ml; $CaCO_3$ 3g; agar-agar 15g; distilled water 800 ml). Inoculation of seedlings was achieved the same way as described for *N. haematococca* f. sp. *piperis.*

Plants were kept under the same conditions and experimental design as mentioned before, and parameters were identical to those for *N. haematococca* f. sp. *piperis.* Observations were made over four months.

Reisolation of the fungi – At the end of the experiments involving inoculation with *N. haematococca* f. sp. *piperis* and *P. palmivora* attempts were made to reisolate both fungi from wilted and symptomless plants. Pieces of roots and stems were surface sterilized for 2 minutes in 5% sodium hypochlorite solution and rinsed twice in sterilized distilled water. For *N. haemato-*

cocca f. sp. *piperis,* pieces of roots and stems were planted on PCNB agar medium (Peptone 10g; KH_2PO_4 1g; $MgSO_4.7H_2O$ 0.5g; streptomycin sulphate 300mg; agar-gar 20g; PCNB – pentachloronitrobenzene 75% wettable powder 1g; distilled water 1,000 ml) (Nash & Snyder, 1962). For *P. palmivora,* pieces of roots and stems were sterilized as before and planted on antibiotic-agar medium (agar-agar 15g; streptomycin sulphate 20mg; distilled water 1,000 ml) (Lee & Varghese, 1974).

RESULTS

M. incognita – Black pepper seedlings infested with *M. incognita,* at all inoculum levels, had significantly (P = 0.05) fewer leaves than controls after six months (Table 1). There was no significant difference between the final height of control plants and those inoculated with 100 second-stage juveniles, but final plant heights in these two treatments were significantly greater than for the other inoculum levels. Initial number of leaves and initial plant height did not differ significantly between treatments. There was a steady increase in the root-knot index corresponding to increase in inoculum levels. The greatest values were recorded in roots of seedlings inoculated with 5,000 and 10,000 second-stage juveniles. These two levels did not differ significantly (P = 0.05) but were significantly different from the other inoculum levels.

Reduction in top fresh weight was significantly detectable (P = 0.05) in seedlings inoculated with 500 juveniles and became more apparent in the inoculum level of 1,000 nematodes, reaching a maximum in seedlings inoculated with 5,000 and 10,000 nematodes. No significant differences were observed in the fresh weight of roots between the treatments, although seedlings infected with higher levels showed a tendency to produce heavier roots. Typical galling and root malformation symptoms were clearly visible in treatments involving inoculation with *M. incognita* juveniles, while control plants exhibited abundant well-formed feeder roots. Total fresh weight decreased with increase in inoculum levels. This trend was significant (P = 0.05) at the 500 nematode level. Total fresh weight continued to decrease reaching the lowest values at the 5,000 and 10,000 nematode levels, which did not differ significantly (Table 1).

For the final nematode population/g root, the number of females without eggs was highest in 5,000 nematode level but not significantly different from 1,000 and 10,000 nematode levels (Table 1). The lowest number of females without eggs was recorded in the 100 level but it did not differ significantly (P = 0.05) from 500 and 1,000 nematode levels. The number of females with eggs was highest and significantly different (P = 0.05) in 10,000 level, compared with all other treatments. In the 100 nematode level, the number of females with eggs was lowest and significantly different from the other inoculum levels. Inoculum levels of 500, 1,000 and 5,000 nematodes per plant produced intermediate values for the number of females with eggs but the 5,000 level was significantly different (P = 0.05) from the 500 and 1,000 levels. Total female populations of *M. incognita*/g root did not differ significantly (P = 0.05) between the inoculum levels of 5,000 and 10,000 nematodes. The same was observed between 500 and 1,000 nematode levels, which did not differ significantly but both were significantly higher than the 100 nematode level. Egg production was significantly higher (P = 0.05) in plants inoculated with 10,000 juveniles, followed by the 5,000 nematode level. At 500 and 1,000 levels, no significant difference was detectable in the egg production/g root. The lowest value was recorded in the 100 nematode level, although no significant difference was observed between this level and 500 nematode level (Table 1).

Table 1 – Influence of different population levels of *M. incognita* on the growth of black pepper six months after inoculation*

Inoculum Levels (No. of Juveniles/ Plant)	No. of Leaves		Plant Height (cm)		Root-knot Index 2	Top Fresh Weight (g)	Root Fresh Weight (g)	Total Fresh Weight (g)	Final nematode population/g Root			
	Initial	Final	Initial	Final					♀ Without Eggs	♀ With Eggs	Total ♀ Population	Eggs
0	7.2 a[1]	12.0 a	11.0 a	20.8 ab	0	6.30 a	2.82 a	9.13 a	0	0	0	0
100	7.4 a	10.0 b	10.9 a	21.0 a	1.2 c	5.86 ab	3.09 a	8.95 a	34.8 c	113.0 d	147.8 c	17573 d
500	7.0 a	8.2 c	10.3 a	17.8 c	1.6 bc	5.04 bc	2.88 a	7.93 b	48.6 bc	140.6 c	189.2 b	21296cd
1,000	7.0 a	8.4 bc	11.4 a	18.3 bc	2.2 b	4.66 c	3.03 a	7.69 b	55.0 abc	173.4 c	234.2 b	26096 c
5,000	7.2 a	6.8 c	10.4 a	13.0 d	4.0 a	2.91 d	3.08 a	5.99 c	72.6 a	317.2 b	389.8 a	47076 b
10,000	7.4 a	6.6 d	9.9 a	12.8 d	4.6 a	2.62 d	3.12 a	5.74 c	60.0 ab	372.0 a	430.0 a	54570 a

* Mean of 5 replicates.

1 Menas in the columns followed by the same letter indicate no significant difference (P = 0.05) according to Duncan's Multiple Range Test.

2 Root-knot index is based on: 1 = none or very light galling; 2 = light galling; 3 = moderate galling; 4 = heavy galling and 5 = very heavy galling.

N. haematococca f. sp. *piperis* – The period between inoculation of *N. haematococca* f. sp. *piperis* and the appearance of wilt symptoms in black pepper seedlings did not differ significantly (P = 0.05) between the eight inoculum levels ranging from 5 x 10^6 to 2 x 10^4 conidia/plant. All these levels were significantly different from 10^4 and 5 x 10^3 conidia levels. The longest period observed between fungal inoculation and wilt symptoms was recorded for the inoculum level of 5 x 10^3 conidia/plant, which was the threshold for pathogenicity (Table 2). Below this inoculum level no plants were killed. Yellowish symptoms and leaf drop typical of naturally infected black pepper plants were observed in plants inoculated with 10^4 and 5 x 10^3 conidia.

The period between fungal inoculation and death of plants presented a slightly different pattern compared to the period between fungal inoculation and appearance of wilt symptoms. Plants inoculated with 5 x 10^6, 10^6 or 5 x 10^5 conidia were killed more rapidly than plants that received lower levels of inoculum. Plants inoculated with 10^5, 5 x 10^4, 4 x 10^4, 3 x 10^4 or 2 x 10^4 conidia presented intermediate values for the parameter measured and there was no significant difference between them. The longest period between fungal inoculation and plant death was observed when plants were inoculated with 5 x 10^3 conidia. This inoculum level was significantly different (P = 0.05) from all the other levels. The second longest value was recorded in plants that received 10^4 conidia level (Table 2).

Table 2 – Influence of different inoculum levels of *N. haemotococca* f. sp. *piperis* in the root and foot rot disease of balck pepper*

Inoculum Levels (No. of Conidia/Plant)	Period Between Fungus Inoculation and Wilt Symptoms (Days)	Period Between Fungus Inoculation and Death (Days)	Plants Wilted and dead (%) **	Isolation of Fungus From Plants (%)**
5 x 10^6	12.8 a[1]	18.0 a	100	100
1 x 10^6	13.6 a	19.2 a	100	100
5 x 10^5	13.4 a	19.0 a	100	100
1 x 10^5	18.9 a	24.8 b	80	100
5 x 10^4	20.2 a	27.2 b	60	80
4 x 10^4	17.2 a	23.6 b	60	60
3 x 10^4	17.9 a	24.9 b	60	60
2 x 10^4	17.6 a	24.7 b	40	60
1 x 10^4	32.1 b	40.2 c	40	40
5 x 10^3	66.1 c	74.7 d	40	40

Mean of 5 replicates adjusted according to the number of missing values (see text).

**Figures not statistically analysed (see text).

[1] Means in the columns followed by the same letter indicate no significant difference (P = 0.05) according to to Duncan's Multiple Range Test.

Percentages of wilted plants decreased with decrease in the inoculum levels. A similar trend was observed for the percentages of fungal isolation from wilted and symptomless plants. *N. haematococca* f. sp. *piperis* was frequently isolated from roots of symptomless plants (Table 2). Both the percentages of wilted and dead plants and the percentages of fungus isolation could not be statistically analysed as they were single figures representing each treatment (Table 2).

Any plant that did not wilt and die in treatments involving the presence of *N. haematococca* f. sp. *piperis* was treated as a missing value. In such cases, the number of replicates was always adjusted to the original number of five by estimating statistically the replacing values according to the number of missing replicates.

P. palmivora – The period between fungus inoculation and wilt symptoms in plants inoculated with *P. palmivora* did not present any great variations between the different inoculum levels. The shortest value was observed for seedlings inoculated with 5 x 10^5 zoospores. This treatment, however, was significantly different (P = 0.05) only from the inoculum levels 2 x 10^4, 2 x 10^3, 10^3 and 5 x 10^2 zoospores, in which wilt symptoms developed slowly (Table 3). Below the 5 x 10^2 level no plants were killed.

Table 3 – Influence of different inoculum levels of *P. palmivora* in the root and foot rot disease of black pepper.*

Inoculum Levels (No. of Zoospores/Plant)	Period Between Fungus Inoculation and Wilt Symptoms (Days)	Period Between Fungus Inoculation and Death (Days)	Plants Wilted and dead (%) **	Isolation of Fungus From Plants (%) **
5 x 10^5	6.0 a[1]	9.8 a	100	100
1 x 10^5	7.6 ab	12.0 ab	100	100
5 x 10^4	8.2 ab	12.8 ab	80	100
4 x 10^4	8.4 ab	13.2 b	60	60
3 x 10^4	8.5 ab	13.7 b	40	40
2 x 10^4	9.4 b	15.2 b	60	60
1 x 10^4	7.4 ab	13.2 b	60	60
5 x 10^3	8.7 ab	13.2 b	60	60
4 x 10^3	8.0 ab	12.7 ab	40	40
3 x 10^3	8.5 ab	14.2 b	40	40
2 x 10^3	10.0 b	14.7 b	40	40
1 x 10^3	9.5 b	14.7 b	40	40
5 x 10^2	9.0 b	14.2 b	40	40

* Mean of 5 replicates adjusted according to the number of missing values (see text).

**Figures not statistically analysed (see text).

[1] Means in the columns followed by the same letter indicate no significant difference (P = 0.05) according to Duncan's Multiple Range Test.

The period between fungus inoculation and plant death did not reveal significant differences ($P = 0.05$) between most of the inoculum levels tested (Table 3). Plants inoculated with 5×10^5, 10^5, 5×10^4 or 4×10^3 zoospores presented the lowest values for this parameter. The inoculum levels of 10^5, 5×10^4 and 4×10^3 zoospores were not significantly different ($P = 0.05$) from the other levels used. None of the inoculum levels tested induced plants to show the typical yellowish symptoms and leaf drop usually observed during natural infecttion of black pepper plants by *P. palmivora*.

Percentages of plants wilted and dead and percentages of fungal isolation from wilted and symptomless plants showed the same trend to decrease with decrease in inoculum levels. *P. palmivora* was less frequently isolated from symptomless plants, in comparison with *N. haematococca* f. sp. *piperis*.

As mentioned for *N. haematococca* f. sp. *piperis*, percentages of wilted and dead plants and fungal isolation from wilted and symptomless plants were not statistically analysed in, the experiment involving *P. palmivora*. These percentages were single values for each treatment, insufficient to permit statistical analysis. Estimation of values for replacing missing replicates of *P. palmivora* were also similar to that previously described.

DISCUSSION

M. incognita – The pathogenic effects of *M. incognita* on black pepper plants were markedly visible when plants were assessed for parasitism six months after inoculation. Final number of leaves decreased as inoculum levels increased, showing a clearly adverse effect due to the nematode infestation. A tendency of black pepper plants to drop leaves when infested with *M. incognita* and *M. javanica* was reported by Winoto (1972). According to Bergeson (1968), if *Meloidogyne* spp. actually impair the root's ability to produce growth substances then the reduced foliage growth observed in plants infested with *Meloidogyne* spp. could be related to the nematode parasitism. Reduction in the final plant height was striking in plants inoculated with 500, 1,000, 5,000 and 10,000 nematodes. Plants inoculated with 100 juveniles of *M. incognita* did not present significant difference from the control plants. Height of black pepper plants affected by *M. incognita* and *M. javanica* was reported by Winoto (1972) and Koshy **et al.** (1979).

Root-knot index increased with increase in inoculum levels, and showed a maximum value at 10,000 nematode level. Despite this apparent correlation, presence of ovigerous females inside roots without any external gall-like symptoms was frequntly observed. Koshy **et al.** (1979) reported an increase of root-knot index with increase in inoculum levels of *M. incognita* in roots of black pepper. Other workers have also reported increase in root galling following increase in inoculum levels of *Meloidogyne* spp. in different plant species (Rao & Israel, Mishra & Gaur, 1981; Reddy, 1981).

Top fresh weight revealed a trend to decrease when nematode levels increased, although plants infested with 100 juveniles had presented top fresh weight similar to the controls. From the initial inoculum level of 500 juveniles top fresh weights were significantly reduced by *M. incognita* parasitism. Koshy **et al.** (1979) reported a decrease in the top fresh weight of black pepper plants starting at the inoculum level of 10 nematodes per plant. The differences observed between our results and those reported by Koshy **et al.** (1979), concerning top fresh weight, can be explained mainly by the period of time that the nematode had to build up its population on black pepper plants. Those workers allowed a period of 12 months between nematode inoculation and assessment of the parasitism, while in our experiment the effects of *M. incognita* upon black pepper plants were evaluated 6 months following inoculation. Also, differences in pathogenicity between the two isolates of *M. incognita* and the reaction of *M. incognita* to different cultivars used would explain the differences in results. In addition, they used stem cuttings whilst the present work was done using seedlings which are far more genetically variable (Gentry, 1955; Holliday & Mowatt, 1963). Reduction in top fresh weight was also reported by Winoto (1972) in black pepper plants infested with *M. incognita* and *M. javanica*.

Root fresh weights of black pepper plants did show any significant difference between the various inoculum levels of *M. incognita* used and the control plants. Identical results were obtained by Winoto (1972) for roots of black pepper plants infected with *M. incognita* and *M. javanica*, although this worker used just one level of inoculum for each nematode. Koshy et al. (1979), on the other hand, detected significant difference in in root fresh weigh of black pepper plants infested with *M. incognita*, for the initial inoculum level of 10 nematodes. Results concerning the root weights in plants infested with *Meloidogyne* spp. are rather contradictory. Wong & Mai (1973) reported a greater root dry weight in lettuce plants infested with *M. hapla* compared to controls. Reddy (1981) did find any significant difference in root fresh weights between various inoculum levels of *M. incognita* in two cultivars of passion fruit. He suggested that top fresh weight is a more reliable parameter to measure than root fresh weight. This suggestion seems to be reasonable considering that galls can compensate for the reduction in number of roots. Despite there being no differences in fresh root weights in the present study the damage caused by *M. incognita* to black pepper roots were evident, and, as a consequence, top fresh weights were seriously affected, and significant reduction in total fresh weight of black pepper plants infested with *M. incognita* occurred with an initial inoculum level of 500 nematodes or above.

The increase of *M. incognita* populations in roots of black pepper was very marked. However, the number of females without eggs was variable and sometimes did not seem to follow the increase in inoculum levels, but the number of females with eggs was a more reliable and measurable parameter and showed a steady increase with increase in inoculum levels. Koshy et al. (1979) reported a peak for final population of *M. incognita* in soil and roots of black pepper at the 10,000 nematodes level, followed by 100,000 level. The highest multiplication factor was recorded in the case of 10 nematodes level and the lowest at 100,000 level. They suggested that the rate of *M. incognita* multiplication was a density dependent phenomenon. A similar trend was observed by Rao & Israel (1972) for *M. graminicola* in rice roots. As discussed by Ellenby (1954), Triantaphylloe & Hirschamnn (1960), and Seinhorst (1970), lower rates of *Meloidogyne* spp. in heavily infested roots are related to competition for space, nutrients, debilitation of roots and increase in density of the nematode population within the roots. In our study such a restraint on the increase of *M. incognita* population in roots of black pepper was not detectable because the highest inoculum level used was 10,000 nematodes.

Total population of *M. incognita* in black pepper roots increased with increase in inoculum levels but was not significantly different between 500 and 1,000 levels, and between 5,000 and 10,000 nematode levels. This may indicate, for the two highest inoculum levels, the beginning of density population phenomenon reported by Rao & Israel (1972) and Koshy et al. (1979). If a longer period of time had been allowed for the multiplication of *M. incognita* in black pepper roots a higher population of this nematode at the initial inoculum level of 5,000 nematodes could have supplanted the 10,000 nematodes level at the end of the experiment.

The results for egg production of *M. incognita* revealed a trend to increase with increase in the inoculum level; the highest number of eggs per gram of roots being recorded at the 10,000 level, which was significantly different from the other levels. Again for the egg production the rate of reproduction of *M. incognita* in roots of black pepper did not exhibit any negative effects due to the initial inoculum levels. For *M. graminicola* in roots of rice, Rao & Israel (1972) reported a lower egg production for initially high inoculum levels, again revealing the influence of population density, although the period between the nematode inoculation and the parasitism assessment had been only 41 days. This could be explained by the short life – cycle of *M. graminicola* under suitable temperature and host plant, and also because females of this nematode tend to lay thier eggs preferentially within the roots, contrary to other species of this genus which lay many egg masses protruding from the root surface. Also in *M. graminicola* most of the hatched juveniles tend to remain within the root tissues, increasing rapidly the population density, whereas species such as *M. incognita* can build up reasonable populations of infective second-stage juveniles in the surrounding soil, relieving the population density inside the infected roots.

The population level of 500 nematodes was the marginal threshold level for producing measurable effects of *M. incognita* on black pepper plants, as observed in the present study. The poor growth of infested plants, the malformation of root systems and the general appearance of nutrient deficiency, revealed the serious damage that *M. incognita* can inflict upon plants of black pepper. Susceptibility of the cultivar Singapura to this harmful nematode seems to be as high as in those black pepper cultivars tested by Winoto (1972) and Koshy **et al.** (1979). Ferraz & Sharma (1979) also detected a marked pathogenicity of *M. incognita* to black pepper plants, cv. Singapura. These results demonstrate the necessity of a more careful way to tackle the problem of root-knot nematode infestation in the Brazilian Amazonian region, where *M. incognita* is widespread in areas suitable for black pepper plantations and Singapura is the only cultivar commercially planted.

N. haematococca f. sp. *piperis* – The period between inoculation of *N. haematococca* f. sp. *piperis* on black pepper plants and appearance of the first wilt symptoms was a parameter dependent upon the fungal inoculum levels. This period was not significantly different from the first eight levels used, beginning with the highest level of 5×10^6 conidia to 2×10^4 conidia level. For 10^4 and 5×10^3 conidia the periods measured were significantly enhanced indicating clearly the separation pont between the two groups of inoculum levels. The marginal threshold for pathogenicity of *N. haematococca* f. sp. *piperis* to black pepper seedlings, regarding the experimental conditions used in this study, was 5×10^3 conidia per plant. Below this inoculum level plants did not show wilt symptoms and were never killed, although the fungus was occasionally isolated from symptomless plants inoculated with levels as low as 4×10^3 and 3×10^3 conidia.

The results obtained from our experiments seem to indicate that the dilution end point (according to Van der Plank (1975) dilution end point is the greatest dilution of inoculum compatile with being reasonably sure of getting infection) was reached at the inoculum level of 3×10^3 conidia per plant, below which reisolation of *N. haematococca* f. sp. *piperis* was not achieved. This demonstrates that infection occurred at this level but not below it. Apparently conidia were able to germinate and penetrate the roots of black pepper but other factors must have affected the continuity of the infection process, leading to a situation of an extremely low rate of fungal growth without apparent symptoms of the fungal presence. As pointed out by Walker (1969) infection implies the establishment of the pathogen within the host following penetration. It does not imply the production of disease symptoms. The survival of *N. haematococca* f. sp. *piperis* in roots of symptomless black pepper plants mainly at the inoculum levels of 4×10^3 and 3×10^3 conidia seems to characterize a long period of incubation. It is also possible that the normal resistance of black pepper seedlings may have affected the continuity of the development of *N. haematococca* f. sp. *piperis* mycelium. The plant resistance apparently was not sufficient to prevent the initial organic contact with the host cells or cytoplasm, as the fungus did invade, but was effective enough to restrain the infection from spread to other tissues and killing the plants, at least during the 4 months period of experimental observations. This situation closely resembles the phenomenon described by Garrett (1970) as "latent infection". However, the presence of *N. haematococca* f. sp. *piperis* in root tissues of symptomless plants and its relatively easy isolation does not fit in the concept of latency discussed by Verhoeff (1974). According to this worker, cases where the fungus can be isolated after surface sterilization cannot be regarded as latent infection but only as part of the normal relationships fungus-plant, which may play a role in fungal survival. He considers as latent infections those quiescent or dormant parasitic relationships which after a time, can change into an acitve one.

Percentages of plants wilted and dead revealed a clear trend to decrease with decrease in inoculum levels. This can be explained based on the variability of the degree of inoculum infectivity, so that the infectivity of some conidia will be too low for successful infection of any of the host individuals under test. Some conidia will fail to germinate on the host site, and others will fail to infect because of disadvantages of position (Garrett, 1970). As for all levels of inoculum some spores are always wasted, the likelihood of successful infection is understandably decreased as the inoculum levels decreased. Decrease in necrotic lesions on aerial parts of black pepper plants inoculated with this fungus and correlated with decrease in inoculum levels was also reported by Albuquerque et al. (1976).

Development of foliar chlorosis and leaf drop, typical of black pepper plants naturally infected with *N. haematococca* f. sp. *piperis*, were visible only in plants inoculated with 10^4 and 5×10^3 conidia. Above these inoculum levels plants wilted and died without displaying chlorosis and leaf drop. Albuquerque & Ferraz (1976) reported the occurrence of yellowish symptoms and leaf drop in seedlings of black pepper inoculated with *N. haematococca* f. sp. *piperis*, by dipping their root systems for five minutes in a suspension of 8×10^4 conidia/ml. This method of inoculation, although far from reproducing natural infections, may have provided a more even distribution of spores around the roots helping the infection spread as a uniform root rot rather than foot rot which kills the plant more rapidly by girdling the stem tissues. Inoculation by pouring the spores in holes around the root systems, as used in this study, seems to favour more the development of foot rot symptoms, apparently giving no chance for the appearance of typical field symptoms of *N. haematococca* f. sp. *piperis* infection for levels higher than 10^4 and 5×10^3 conidia.

The presence of typical symptoms seems to be related to the slower action of fungal toxins. As young tissues of black pepper plants always revealed a greater susceptibility to *N. haematococca* f. sp. *piperis,* in comparison with older ones, toxins acted rapidly without permitting infected plants to develop the typical symptoms of the disease. Action of fungal toxins and degrading enzymes in root and foot rot diseases have been discussed by Wood (1967) and Garrett (1970).

The period between fungal inoculation and death of plants showed a slight difference compared with the period between fungal inoculation and wilt symptoms, although there was the same trend for the period to increase as the inoculum levels decreased. Once more, the longest periods were detected for the levels of 10^4 and 5 x 10^3 conidia. Periods for the appearance of wilt symptoms in plants infected with *N. haematococca* f. sp. *piperis* were similar to those reported by Albuquerque & Ferraz (1976). Some minor differences in results could be explained by the utilization of a different fungal isolate or the experimental conditions in which the tests were conducted.

The pathogenic capability of *N. haematococca* f. sp. *piperis* to seriously infect and kill plants of black pepper, as confirmed in the present work, agrees with the results reported by Albuquerque & Conduru (1971) and Albuqueruqe & Ferraz (1976) and it emphasizes the threat posed by this fungus to black pepper plantations in Brazil.

P. palmivora – Pathogenicity tests with *P. palmivora* on black pepper plants have been conducted by many workers but comparisons between results are almost impossible because different types and inoculum concentrations have been used (Holliday & Mowatt, 1963; Leather, 1967; Turner, 1971, 1973; Alconero **et al.**, 1972). A comprehensive report on the subject has been provided recently by Kueh & Khew (1982). They compared different concentrations of inocula represented by mycelial, sporangial and zoosporic suspensions, and identified suitable inoculum levels for each type of propagule used. As for zoospores, concentrations of 10^3, 10^4, 10^5 and 10^6 zoospores/ml did not differ significantly between them but all were significantly different from 10^7 zoospores/ml concerning the percentage of root destruction. Despite the useful informations provided, Kueh & Khew (1982) did not establish the threshold for any of the inocula used.

In our work, eight-month old seedlings of the cultivar Singapura were infected and killed with inoculum levels ranging from 5 x 10^5 to 5 x 10^2 zoospors, without great differences concerning the period between fungal inoculation and wilt symptoms and period between fungal inoculation and plant death. The 5 x 10^2 zoospores lavel was the marginal threshold for *P. palmivora* to infect roots and kill the plants, in the experimental and environmental conditions under which the tests were undertaken. Inoculum levels below 5 x 10^2 zoospores were not able to infect roots, as confirmed during attempts to reisolate the fungus from roots of black pepper seedlings at the end of the experiment.

P. palmivora was reisolated from all plants inoculated and killed by this fungus, but fungal isolation from symptomless plants was low compared to *N. haematococca* f. sp. *piperis. P. palmivora* was isolated only from symptomless plants inoculated with 5 x 10^4 zoospores. The possible explanation for the occurrence of hyphae of this fungus in roots of plants without apparent disease symptoms could be the same as those mentioned for *N. haematococca* f. sp. *piperis.* Contrary to many pathogenic fungi, *P. palmivora* is not easily isolated from infected plant tissues or infested soil, requiring usually complex culture media with antibiotics to restrain the growth of secondary organisms (Eckert & Tsao, 1962; Tsao & Ocana, 1969; Tsao, 1970; Lee & Varghese, 1974). In this study the medium used to isolate *P. palmivora* was simply agar plus 20 mg of streptomycin sulphate/litre of medium and a higher percentage of resisolation of this fungus from roots of symptomless plants could have been achieved if a more complex culture medium had been used. On the other hand, since the experiments were conduceted in autoclaved soil, presence of competitors during isolation of *P. palmivora* was always low.

Percentages of plants wilted and dead decreased as the inoculum levels decreased. This trend could be explained in the same manner as discussed previously for inocula of *N. haematococca* f. sp. *piperis.* However, there are marked differences between the two types of inocula used. Zoospores of *P. palmivora* are motile cells which after establishing contact with the root surface become immobile, encyst and initiate penetration. In additon, zoospores are induced to encyst by many factors such as collisions with other zoospores or solid surfaces, changes in hydrogen ion concentration or temperature, variations in water current (rheotaxis) and variations in nutrients or ions (Mitchell **et al.**, 1978). All these factors make zoospores's behaviour more complex compared to static spores like conidia.

Increase in infection and mortality of tobacco plants following increase in concentration of zoospores of *P. parasitica* var. *nicotianae* have been reported by Gooding & Lucas (1959), and by Mitchell **et al.** (1978) for watercress plants inoculated with *P. cryptogea.* Infection in cotyledons of alfalfa seedlings by P. megasperma was achieved with inoculum level as low as 9 zoospores. Fewer plants were severely diseased when less than 5 zoospores were applied to each cotyledon. Ramirez & Mitchell (1975) observed that infection of papaya seedlings by *P. palmivora* began at the inoculum level of 10^3 zoospores/plant but 10^4 zoospores were required to initiate mortality. The results we have obtained from the experiments conducted either with *N. haematococca* f. sp. *piperis* or with *P. palmivora* provide the first informations concerning the numbers of conidia and zoospores necessary to cause infection and/or wilt and death of black pepper seedlings under glasshouse conditions.

As previously discussed for *N. haematococca* f. sp. *piperis,* the problem of black pepper infection by *P. palmivora* in the Brazilian Amazonian region deserves special attention and planning in order to find out suitable and mainly economic control measures. Control should preferentially be through the utilization of genetic resistance transferred from other cultivars and species of black pepper, if found, or even from wild species of the genus *Piper* or other related genera of the same family. The production and distribution of new cultivars with genetic resistance to both fungi, which is the main objective of the Brazilian Breeding Programme, is potantially the most economic control but it will probably be a few years before it can be shown whether any of the cultivars will be suitable for replacing the high-yielding though susceptible cultivar Singapura.

LITERATURE CITED

ALBUQUERQUE, F.C. Podridao das raízes e do pê da pimenta do reino. Circular 5, Instituto Agronomico do Norte, Belêm, 45 pp. 1961.

ALBUQUERQUE, F.C. Podridão do pe da pimenta do reino (*Piper nigrum* L.) causado por *Phytophthora palmivora* (Butler) Butler. Atas do Instituto de Micologia. Recife 3: 468-491. 1966.

ALBUQUERQUE, F.C. & CONDURU, J.M.P. A cultura da pimenta-do-reino na regiao Amazonica. Belém, IPEAN. Serie Fitotecnica, Vol. 2, 149 pp. 1971.

ALBUQUERQUE, F.C. & FERRAZ, S. Caracteristicas morfologicas e fisiologicas de *Nectria haematococca* f. sp. *piperis* e sua patogenicidade a pimenta-do-reino (*Piper nigrum* L.). Experientae 22: 133-151. 1976.

ALBUQUERQUE, F.C.; FERRAZ, S. & SEDIYAMA, C.S. Influência da tecnica de inoculação e da concentração de esporos na patogenicidade de *Nectria haematococca* f. sp. *piperis* sobre pimenta-do-reino. Experintae 22: 165-174. 1976.

ALCONERO, R.; ALBUQUERQUE, F.C.; ALMEYDA, N. & SANTIAGO, A.G. Phytophthora foot rot of black pepper in Brazil and Puerto Rico. Phytopathology 62: 144-148. 1972.

ARENS, M.L. & RICH, J.R. Yield response and injury levels of *Meloidogyne incognita* and *M. javanica* on the susceptible tobacco "McNair 944". Journal of Nematology 13: 196-201. 1981.

AYYAR, P.K. A preliminary note on the root gall nematode *Heterodera radicicola* and its economic importance in Sourth India. Madras Agricultural Journal 14: 113-118. 1926.

BERGESON, G.B. Evaluation of factors contributing to the pathogenicity of *Meloidogyne incognita*. Phytopathology 58: 49-53. 1968.

DHAWAN, S.C. & SETHI, C.L. Observations on the pathogenicity of *Meloidogyne incognita* to eggplant and on relative susceptibility of some varieties to the nematode. Indian Journal of Nematology 6: 39-46. 1976.

ECKERT, J.W. & TSAO, P.H. A selective antibiotic medium for isolation of *Phytophthora* and *Pythium* from plant roots. Phytopathology 52: 771-777. 1962.

ELGIN, J.H. Jr.; GRAY, F.A.; PEADEN, R.N.; FAULKNER, L.R. & EVANS, D.W. Optimum inoculum levels for screening alfalfa seedlings for resistance to northern root-knot nematode in a controlled environment. Plant Disease Reporter 57: 657-660. 1973.

ELLENBY, C. Environmental determination of sex ratio of a plant parasitic nematode. Nature 174: 1016. 1954.

FERRAZ, E.C.A. & SHARMA, R.D. Interaçao e patogenicidade do *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 e o *Rotylenchulus reniformis* Linford & Oliveira, 1940 na pimenteira-do-reino. Revista Theobroma 9: 45-53. 1979.

FREIRE, F.C.O. & MONTEIRO, A.R. Nematodes associated with *Piper nigrum* in the Amazon region. Resumos dos trabalhos cientificos e conferencias, III Reuniao Brasileira de Nematologia, Sociedade Brasileira de Nematologia, Escola Superior de Agricultura, Mossoro, 1978. Mossoro, RN, Coleçao Mossoroense, Vol. 62, 82.

GARRETT, S.D. Pathogenic Root-Infecting Fungi. Cambridge University Press, 294 pp. 1970.

GENTRY, H.S. Introducing black pepper into America. Economic Botany 9: 256-268. 1955.

GOODING, G.V. & LUCAS, G.B. Effect of inoculum on the severity of tobacco black shank. Phytopathology 49: 274-276. 1959.

HOLLIDAY, P. & MOWATT, W.P. Foot rot of *Piper nigrum (Phytophthora palmivora).* Commonwealth Mycological Institute, Kew, England, Phytopahtological Paper No. 5, 62 pp. 1963.

HOLLIDAY,`P. A wilt of *Piper nigrum* in Brazil. Commonwealth Phytopathological News Part 5, 4. 1965.

ICHINOHE, M. Infestation of black pepper vines by the root-knot nematode, *Meloidogyne incognita,* at Tome-acu Para, Brazil. Japanese Journal of Nematology 5: 36-40. 1975.

JACOB, A.J. & KURIAN, J. Survey of nematodes associated with pepper in Kerala. Agricultural Research Journal of Kerala 17: 270-271. 1979.

KANNWISCHER, M.E. & MITCHELL, D.J. Relationships of numbers of spores of *Phytophthora parasitica* var. *nicotianae* to infection and mortality of tobacco. Phytopathology 71: 69-73. 1981.

KLIEJUNAS, J.T. & KO, W.H. Effect of motility of *Phytophthora palmivora* zoospores on disease severity in papaya seedlings and substrate colonization in soil. Phytopathology 64: 426-428. 1974.

KOSHY, P.K.; PREMACHANDRAN, D.; SOSAMMA, V.K. & PREMKUMAR, T. Effect of *Meloidogyne incognita* population on black pepper. Indian Phytopathology 32: 221-225. 1979.

KUEH, T.K. & KHEW, K.L. A screening technique useful in selecting for resistance in black pepper to *Phytophthora palmivora.* Malaysian Agricultural Journal 52: 37-45. 1982.

LEATHER, R.I. The occurrence of a *Phytophthora* root and leaf disease of black pepper in Jamaica. F.A.O. Plant Protection Bulletin 15: 15-16. 1967.

LEE, B.S. & VARGHESE, G. Studies on tl genus *Phytophthora* in Malaysia. I. Isolation techniques, comparative morphology and physiology and reaction to antibiotics. Malaysian Agricultural Research 3: 13-21. 1974.

LORDELLO, L.G.E. & SILVA, H.M. Um nematoide nocivo a pimenteira-do-reino. Revista de Agricultura, Piracicaba. 49: 165-166. 1974.

MISHRA, S.D. & GAUR, H.S. Effect of indidivual and concomitant inoculation with *Meloidogyne incognita* and *Rotylenchulus reniformis* on the growth of black gram (*Vigna mungo*). Indian Journal of Nematology 11: 25-28. 1981.

MITCHELL, D.J. Relationships of inoculum levels of several soilborne species of *Phytophthora* and *Pythium* to infecntion of several hosts. Phytopathology 68: 1754-1759. 1970.

NADAKAL, A.M. Studies on plant parasitic nematodes of Kerala. III. An additional list of plants attacked by root-knot nematodes, *Meloidogyne* sp. Journal of Bombay National History Society 61: 467-469. 1964.

NAMBIAR, K.K.N. & SARMA, Y.R. Wilt diseases of black pepper. Journal of Plantation Crops 5: 92-103. 1977.

NASH, S.M. & SNYDER, W.C. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. Phytopathology 52: 567-572. 1962.

RAMIREZ, B.N. & MITCHELL, D.J. Relationships of density of chlamydospores and zoospores of *Phytophthora palmivora* in soil to infection of papaya. Phytopathology 65: 780-785. 1975.

RAO, Y.S. & ISRAEL, P. Influence of inoculum density on the final population of root-knot nematode (*Meloidogyne graminicola*) in rice. Indian Journal of Nematology 2: 72-76. 1972.

RAUT, S.P. & SETHI, C.L. Studies on the pathogenicity of *Meloidogyne incognita* on soybean. Indian Journal of Nematology 10: 166-174. 1980.

RAUT, S.R. Effect of initial inoculum levels of *Meloidogyne incognita* on plant growth and rhizobial nodulation on mungbean. Indian Phytopathology 33: 351-353. 1980.

REDDY, P.P. Pathogenicity of *Meloidogyne incognita* to passion fruit. Indian Journal of Nematology 11: 80-81. 1981.

RUPPEL, E.G. & ALMEYDA, N. Susceptibility of native pepper species to the collar rot pathogen of black pepper in Puerto Rico. Plant Disease Reporter 49: 450-551. 1965.

SEINHORST, J.W. Dynamics of populations of plant parasitic nematodes. Annual Review of Phytopathology 8: 131-156. 1970.

SHARMA, R.D. & LOOF, P.A.A. Nematodes of the cocoa region of Bahia, Brazil. IV. Nematodes in the rhizospheres of pepper (*Piper nigrum* L.) and clove (*Eugenia caryophyllata* Thunb.) Revista Theobroma 4: 26-32. 1974.

SHEW, H.D. & BENSON, D.M. Effect of inoculum density of *Phytophthora cinnamomi* on root rot of Fraser fir. Phytophthora Newsletter No. 7: 20. 1979.

SUNDARARAJU, P.; KOSHY, P.K. & SOSAMMA, V.K. Plant parasitic nematodes associated with spices. Journal of Plantation Crops 7: 15-26. 1979.

TRIANTAPHYLLOU, A.C. & HIRSCHMANN, H. Post-infection development of *Meloidogyne incognita* Chitwood, 1949 (Nematoda: Heteroderidae). Anales de L'Institut Phytopathologique Benaki 3: 3-11. 1960.

TSAO, P.H. & OCANA, G. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. Nature 223: 636-638. 1969.

TSAO, P.H. Selective media for isolation of pathogenic fungi. Annual Review of Phytopathology 8: 157-186. 1970.

TURNER, G.J. Resistance in Piper species and other plants to infection by *Phytophthora palmivora* from *Piper nigrum.* Transactions of the British Mycological Society 57: 61-66. 1971.

TURNER, G.J. Pathogenic variation in isolates of *Phytophthora palmivora* from *Piper nigrum.* Transactions of the British Mycological Society 60: 583-585. 1973.

VAN DER PLANK, J.E. Genetic and Molecular Basis of Plant Pathogenesis. Springer – Verlag Berlin, Heidelberg, 167 pp. 1978.

VENKITESAN, T.S. On the occurrence of plant parasitic nematodes associated with different crops in Cannanore District, Kerala. Agricultural Research Journal of Kerala 10: 179-180. 1972.

VERHOEFF, K. Latent infections by fungi. Annual Review of Phytopathology 12: 99-110. 1974.

WALKER, J.C. Plant Pathology. McGraw – Hill Inc., London, 819 pp. 1969.

WINOTO, R.S. Effect of *Meloidogyne* species on the growth of *Piper nigrum* L. Malaysian Agricultural Research 1: 86-90. 1972.

WONG, T.K. & MAI, W.F. Pathogenicity of *Meloidogyne hapla* to lettuce as affected by inoculum level, plant age at inoculation and temperature. Journal of Nematology 5: 126-129. 1973.

WOOD, R.K.S. Physiological Plant Pathology. Blackwell Scientific Publications, Oxford and Edinburgh, 570 pp. 1967.